

# **VIROTHERAPY**

# **A MODERN CANCER TREATMENT**

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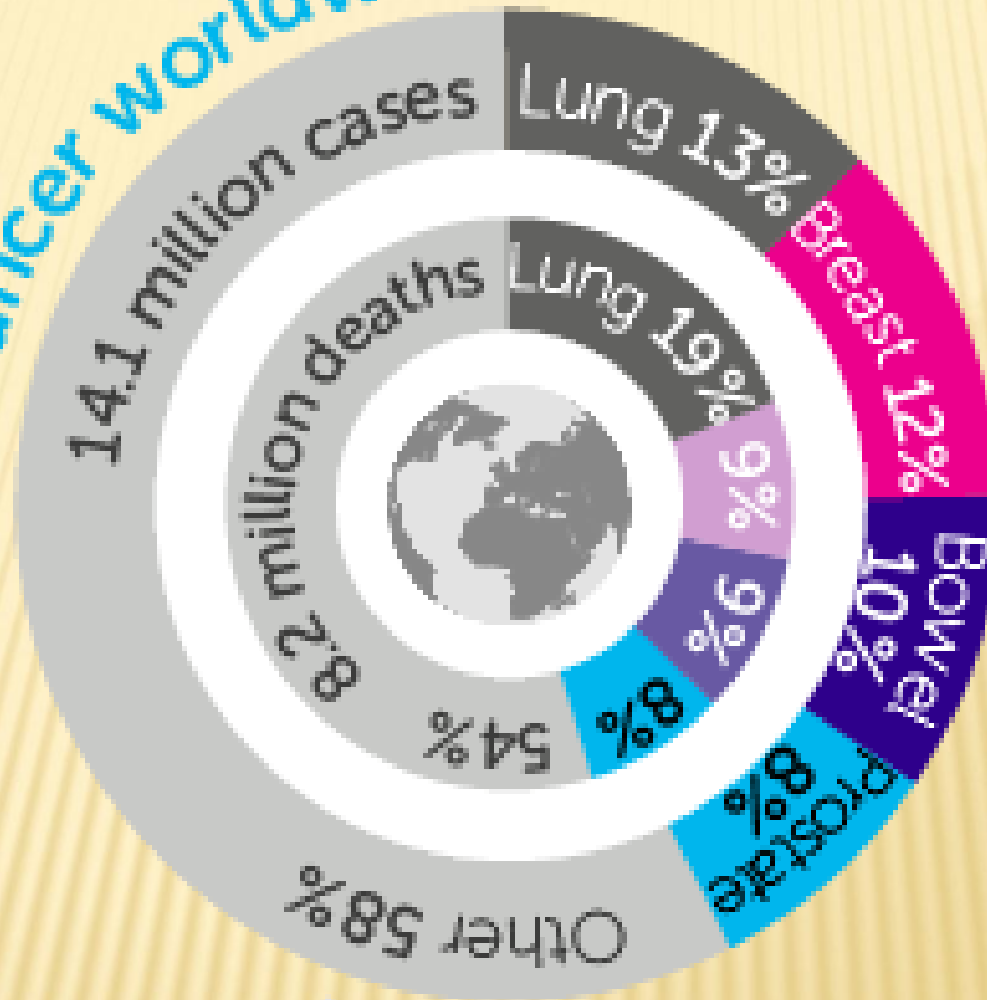
*in the name of god*



# CANCER FACTS

- ✖ Cancers figure among the leading causes of morbidity and mortality worldwide, with **approximately 14 million new cases and 8.2 million cancer related deaths** in 2012.
- ✖ The number of new cases is expected to **rise by about 70% over the next two decades.**
- ✖ Cancer causing **viral infections** such as HBV/HCV and HPV are responsible for **up to 20%** of cancer deaths in low- and middle-income countries (2).
- ✖ It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next 2 decades.
- ✖ The financial costs of cancer have been estimated at **\$1.2 trillion dollars** per year

# Cancer worldwide



Liver



Stomach

2012

# CCANCER TREATMENTS

- × Surgery
- × Chemotherapy
- × Radiation Therapy
- × Targeted Therapy
- × Immunotherapy
- × Stem Cell Transplant
- × Photodynamic Therapy
- × Lasers in Cancer Treatment





# CLASSIC TREATMENTS SIDE EFFECTS

- ✗ damage to normal cells causes side effects, Normal cells usually recover when therapy is over
- ✗ The fast-growing, normal cells most likely to be affected are:
  - blood cells forming in the bone marrow
  - cells in the digestive tract (mouth, stomach, intestines, esophagus)
  - reproductive system (sexual organs)
  - hair follicles.



# CLASSIC TREATMENTS SIDE EFFECTS

- × Fatigue
- × Nausea & Vomiting
- × Pain
- × Hair Loss
- × Anemia
- × Infection
- × Blood Clotting Problems
- × Mouth, Gum and Throat Problems
- × Diarrhea and Constipation
- × Nerve and Muscle Effects
- × Effects on Skin and Nails
- × Kidney and Bladder Effects
- × Flu-Like Symptoms
- × Fluid Retention
- × Effects on Sexual Organs and Sexuality



# TARGETED THERAPY IN CANCER

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- ✖ Targeted therapy is a newer type of cancer treatment that uses drugs or other substances to more precisely identify and attack cancer cells
- ✖ ...usually little damage to normal cells.
- ✖ Targeted therapy is a growing part of many cancer treatment regimens.



# Targeted Therapy

Hormone therapies

Signal transduction  
inhibitors

Apoptosis inducers

Gene expression  
modulators

Angiogenesis inhibitors

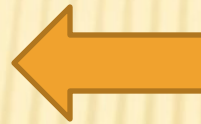
oncolytic virus therapy

gene therapy

Monoclonal antibodies that  
deliver toxic molecules

Cancer vaccines

Immunotherapies



# WHAT IS VIROTHERAPY?

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- ❖ Virotherapy is an experimental form of cancer treatment using biotechnology to convert viruses into cancer-fighting agents by reprogramming viruses to attack cancerous cells, while healthy cells remained relatively undamaged...
- ❖ Viral oncotropism is a term used to define the ability or property of viruses to find and destroy ( oncolysis ) malignant tumour cells, without harming healthy cells.
- ✕ **Modern Approach to Cancer Treatment Without Borders**



# VIROTHERAPY ADVANTAGES:

Virotherapy has many advantages compared to chemotherapy and radiotherapy:

- ✗ Virotherapy induces **selective oncolysis** of sensitive tumours
- ✗ Virotherapy has **little or no side effects**.
- ✗ Virotherapy has a **high therapeutic index**, in some cases even 10000:1 (10000 tumour cells break down in relation to 1 healthy cell).
- ✗ Virotherapy **activates the immune system**.
- ✗ The products of tumour cell breakdown are **excreted quicker**.

# VIROTHERAPY SUBCATEGORIES

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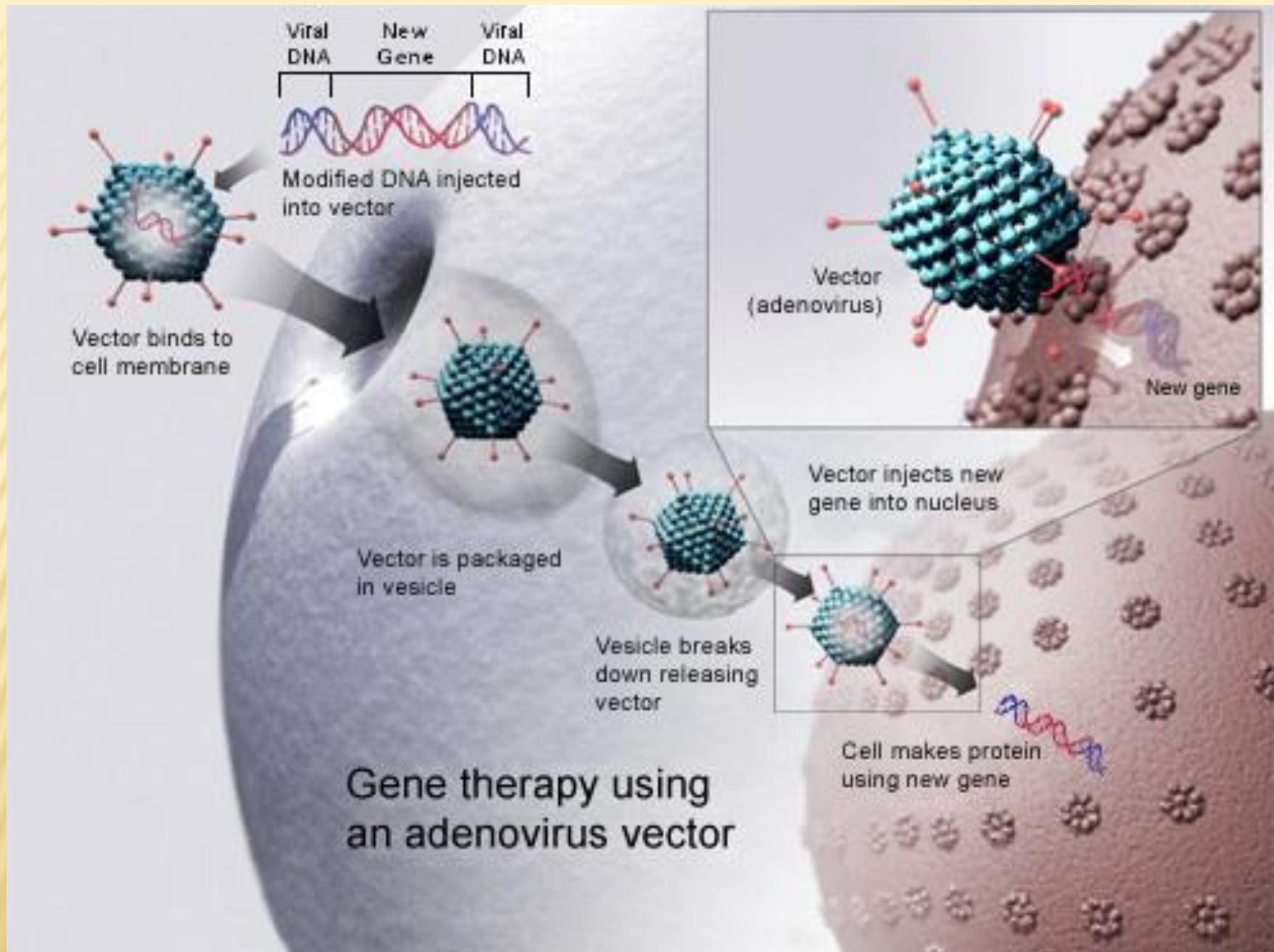
- ✗ There are 4 main branches of virotherapy:
  - ✓ **anti-cancer oncolytic viruses**
  - ✓ Viral vectors for gene therapy
  - ✓ Viral immunotherapy
  - ✓ Drug Delivery (virosom)
- ✗ virotherapy can also refer more broadly to the **use of viruses to treat certain medical conditions** (like Infections) by killing pathogens...



# VIRAL GENE THERAPY

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- uses non-replicating viruses to deliver therapeutic genes to cells with genetic malfunctions.
- first conceptualized in 1972.
- first FDA-approved in 1990
- By January 2014, about 2,000 clinical trials had been conducted or had been approved
- uncontrolled delivery of a gene and Immune responses to viral therapies is most problems in this method.
- ProSavin is one of a number of therapies in the Lentivector. It delivers to the brain the genes for three enzymes important in the production of dopamine, a deficiency of which causes Parkinson's disease





# VIRAL IMMUNOTHERAPY

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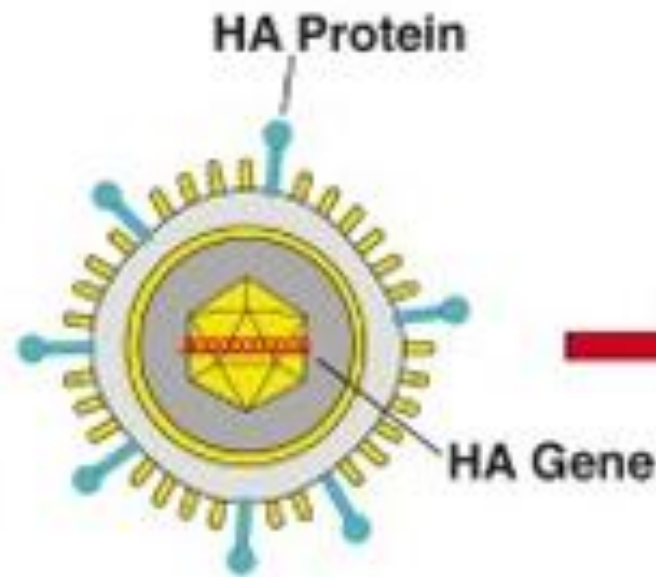
- viral immunotherapy uses genetically engineered viruses to present a specific antigen to the immune system.
- That antigen could be from any species of **virus, bacteria** or even human disease antigens, for example **cancer antigens...**
- Trovax is an immunotherapy that uses a pox-virus bearing the tumour antigen 5T4, to induce an immune response against a variety of cancer types.
- Ebola vaccine production by this method is Under investigation...



**Virus Vector**  
(e.g. adenovirus,  
alphavirus)



**Influenza Virus**  
**Gene(s)**



**Virus Vector Vaccine**  
**Expressing HA Protein**



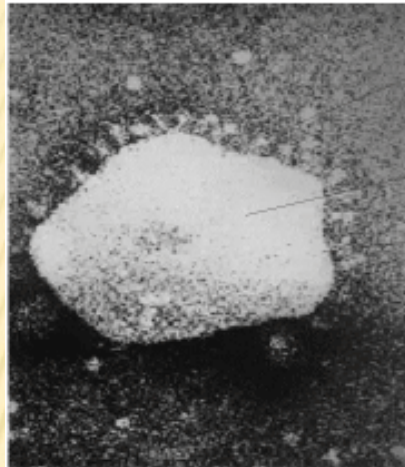
**Vaccine**  
**Vial**



# VIROSOME DRUG DELIVERY

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- ✗ Virosomes is A novel strategy for Drug Delivery and Targeting..
- ✗ virosomes are biocompatible, biodegradable, nontoxic, and non-autoimmunogenic, attempts have been made to use them as vaccines or adjuvants as well as delivery systems for drugs, nucleic acids, or genes for therapeutic purposes.
- ✗ To enhance the efficiency of Drug delivery by the introduction of molecules directly into cells, virosomes have been developed by combining liposomes with fusiogenic viral envelope proteins.

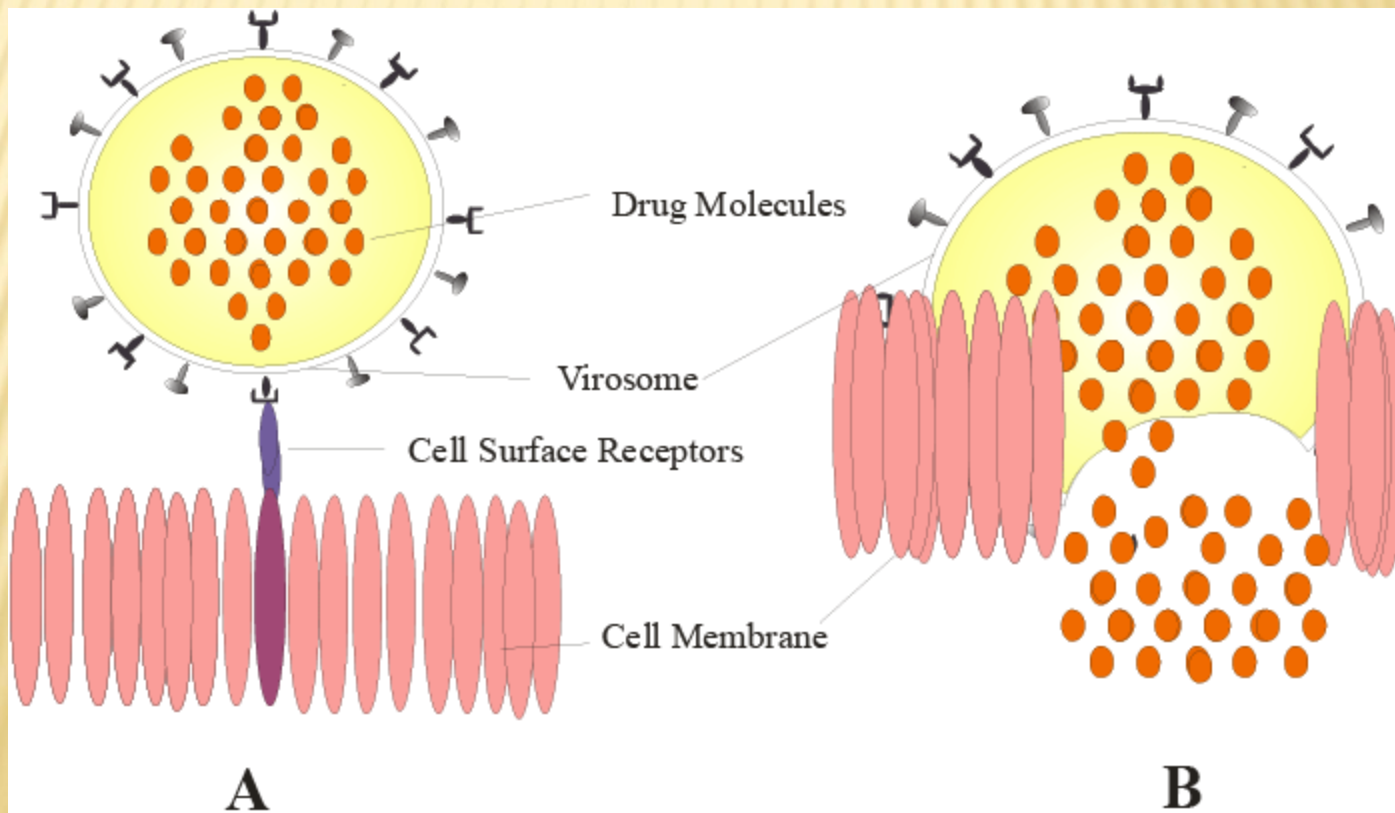
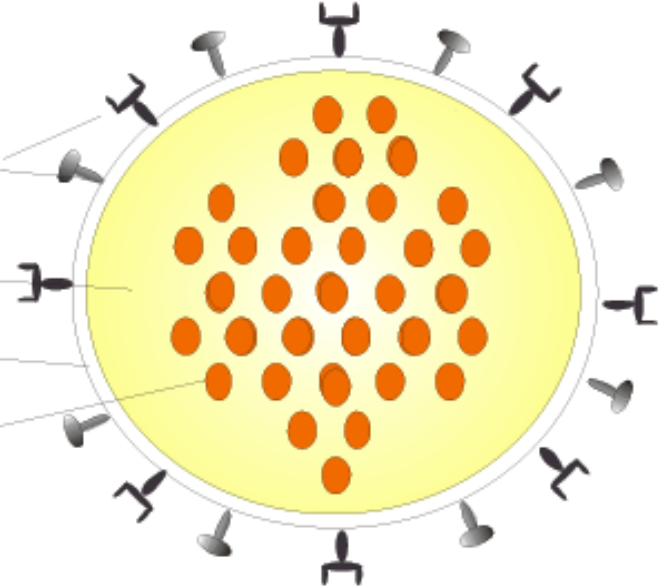


Viral Surface Glycoproteins

Virosome Cavity

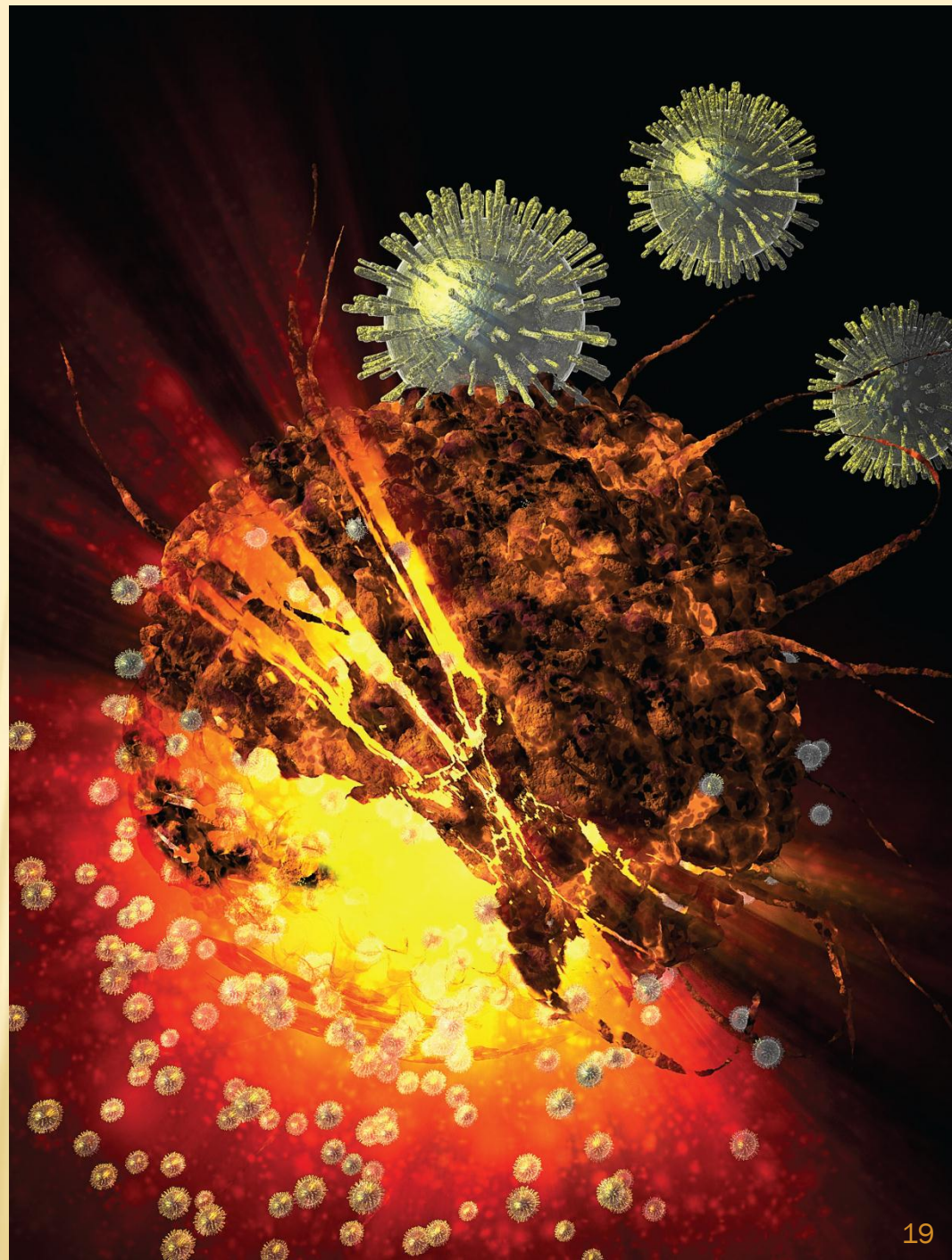
Lipid Bilayer

Therapeutic Agent



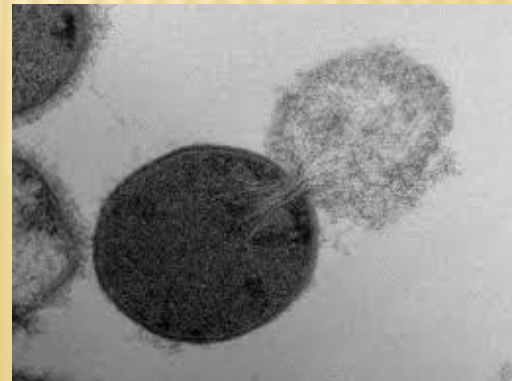


# Oncolytic virotherapy



# ONCOLYTIC VIROTHERAPY

- ✗ The use of (oncotropic / oncolytic) viruses in oncology is called Oncolytic virotherapy.
- ✗ modify existing viruses to create new oncolytic viruses that are less susceptible to immune suppression while more specifically targeting particular classes of cancer cells.
- ✗ similarly as radiotherapy and chemotherapy, causes cell breakdown (cytolysis).





# HISTORY

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In 1904 in Italy a woman confronted two life-threatening events: first, diagnosis with cancer of the uterine cervix, then a dog bite.

Doctors delivered the rabies vaccine for the bite, and subsequently her “enormously large” tumor disappeared. The woman lived cancer-free until 1912.

Soon thereafter several other Italian patients with cervical cancer also received the vaccine (a live rabies virus that had been weakened).

As reported by Nicola De Pace in 1910, tumors in some patients shrank, presumably because the virus somehow killed the cancer. All eventually relapsed and died, however.

# HISTORY

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- ✗ In the 1940s and 1950s, studies were conducted in animal models to evaluate the use of viruses in the treatment of tumours and some of the earliest human clinical trials with oncolytic viruses were started...

But Stopped Until 1990s, because:

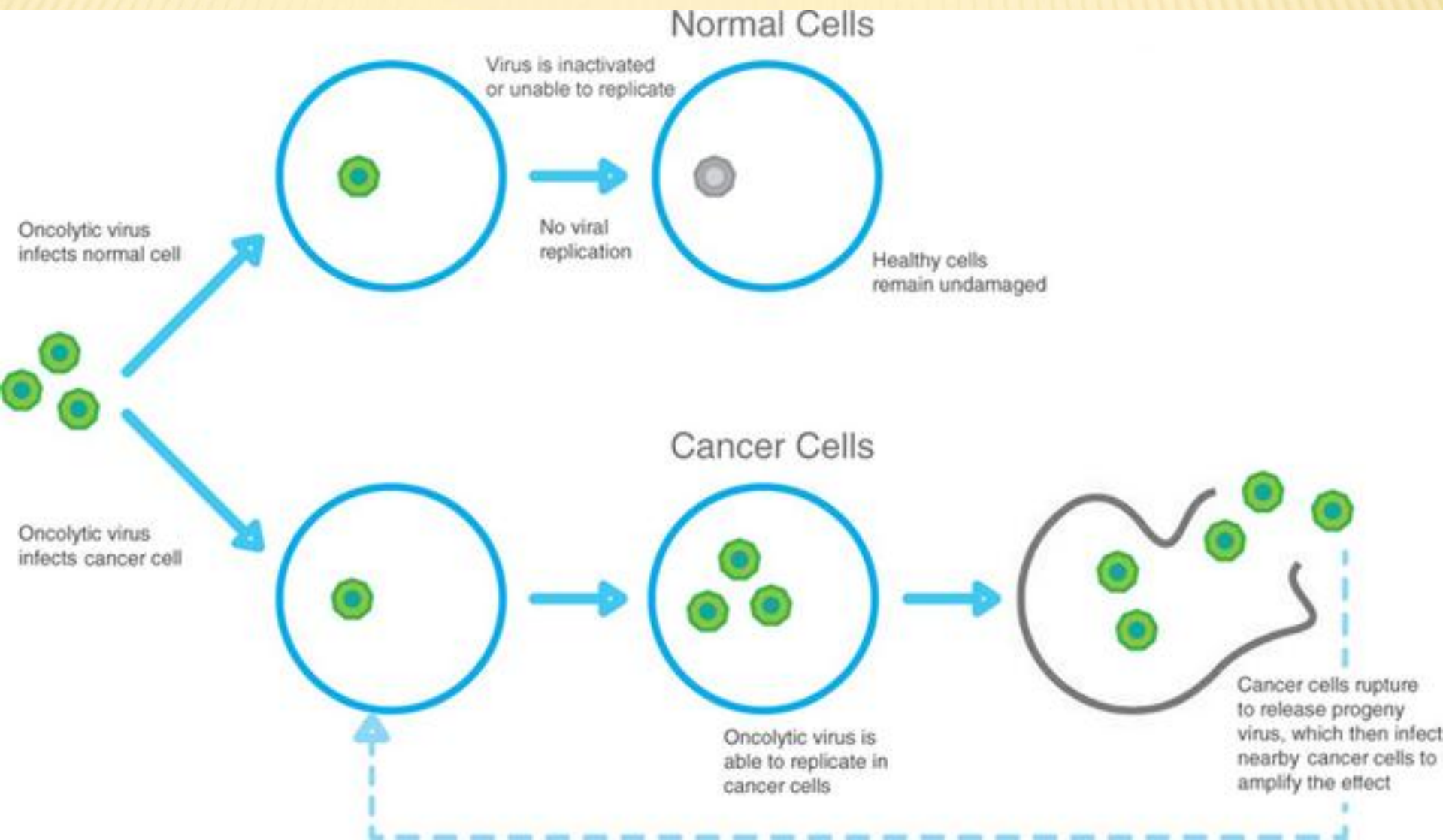
- ✗ uncertainty about its mechanisms and how to use viruses to achieve cures.
- ✗ a dearth of tools with which to engineer more effective viral strains
- ✗ the habitual reluctance of physicians to infect patients with pathogens.



# WHAT IS AN ONCOLYTIC VIRUS

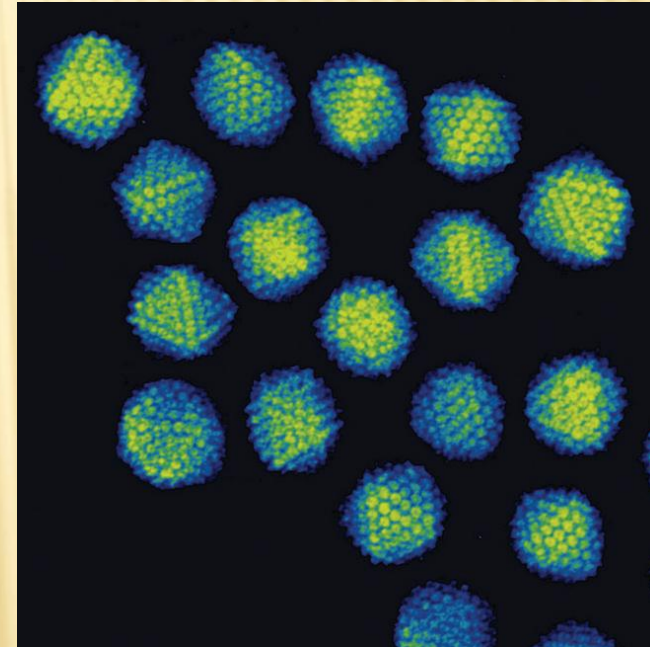
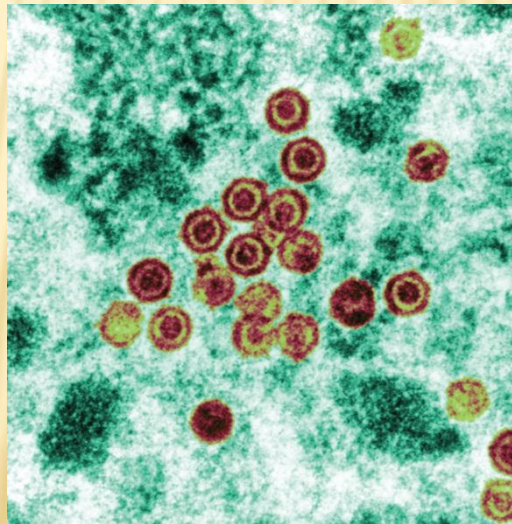
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- ✗ An **oncolytic virus** is a virus that preferentially infects and kills cancer cells.
- ✗ the infected cancer cells are destroyed by lysis, they release new infectious virus particles to help destroy the remaining tumour...
- ✗ Oncolytic viruses are thought not only to cause direct destruction of the tumour cells, also to stimulate host anti-tumour immune responses.



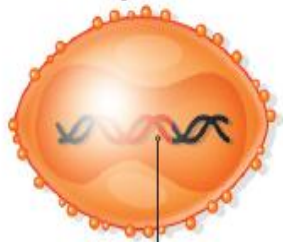
# PROGRAMMABLE BIOLOGICAL MACHINES

- ✗ Herpes simplex virus
- ✗ Adenovirus
- ✗ Measles virus
- ✗ Reovirus
- ✗ Vaccinia virus
- ✗ ....





Oncolytic virus



Gene for antigen

Researchers can insert genes for tumor antigens—molecules that elicit immune responses—into viruses. Infected tumor cells then produce the antigens, enhancing immune activity against such cells.

## HOW IT WORKS

# How Oncolytic Viruses Destroy Tumors

Not all viruses attack cancer cells, but some are especially good at targeting tumors and ignoring healthy tissues. Researchers are learning how to modify these viruses (*inset at left*) to awaken a stronger immune response against the tumor (*below*). Ideally, this approach would be paired with new treatments (*not shown*) that block a tumor's ability to suppress the immune system.

Blood vessel    Replicating virus    Tumor cell lysis (dark gray)

Infected tumor cell

Tumor cells

Oncolytic virus

### Direct Killing (Lysis) of Cancer Cells

Once inside a cancer cell, the virus forces it to make many more viruses. This new viral army charges out of the infected cell, killing it, and seeks out new cancer cells to infect. Or the viruses may simply reprogram infected tumor cells to self-destruct in a process known as apoptosis.



### Vascular Collapse

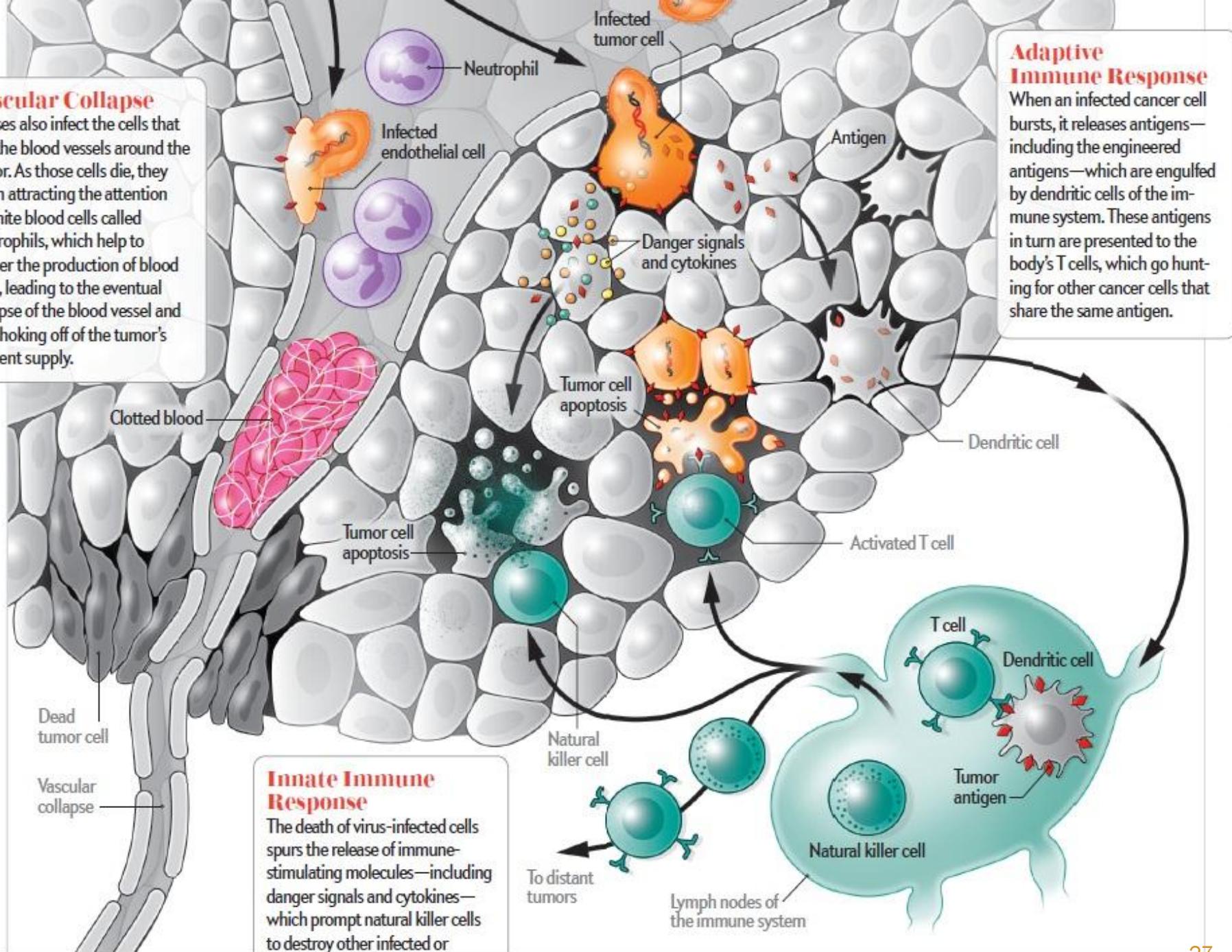
Viruses also infect the cells that line the blood vessels around the tumor. As those cells die, they begin attracting the attention of white blood cells called neutrophils, which help to trigger the production of blood clots, leading to the eventual collapse of the blood vessel and the choking off of the tumor's nutrient supply.

### Adaptive Immune Response

When an infected cancer cell bursts, it releases antigens—including the engineered antigens—which are engulfed by dendritic cells of the immune system. These antigens in turn are presented to the body's T cells, which go hunting for other cancer cells that share the same antigen.

### Innate Immune Response

The death of virus-infected cells spurs the release of immune-stimulating molecules—including danger signals and cytokines—which prompt natural killer cells to destroy other infected or noninfected tumor cells.





# HOW... ?!

- ✗ advances in molecular engineering has allowed for manipulation of the viral genome to both make them safer by deleting viral genes involved in pathogenesis and by insertion of novel transgenes to enhance antitumor activity...

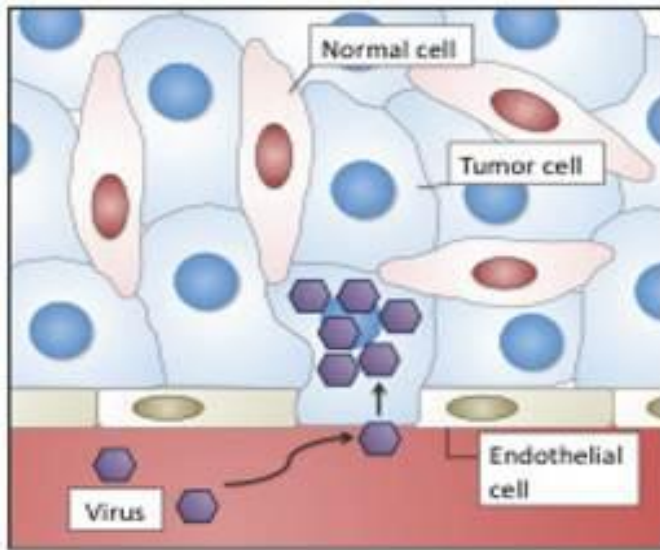




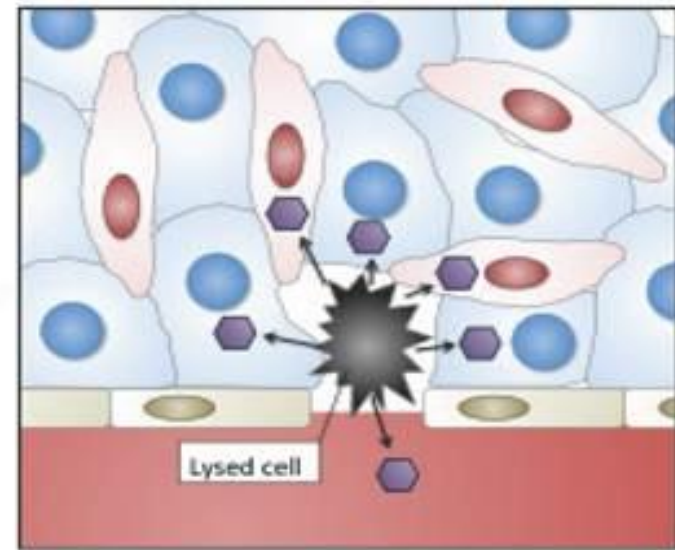
# Tumour Targeting concept



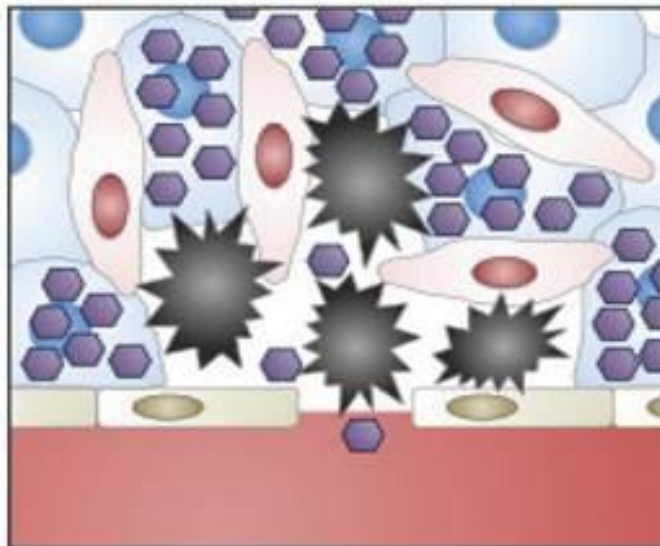
Using Antigenic And Internal differences between healthy and cancerous cell to to design a specific lytic virus that Attach or replicate in cancerous cells only.....



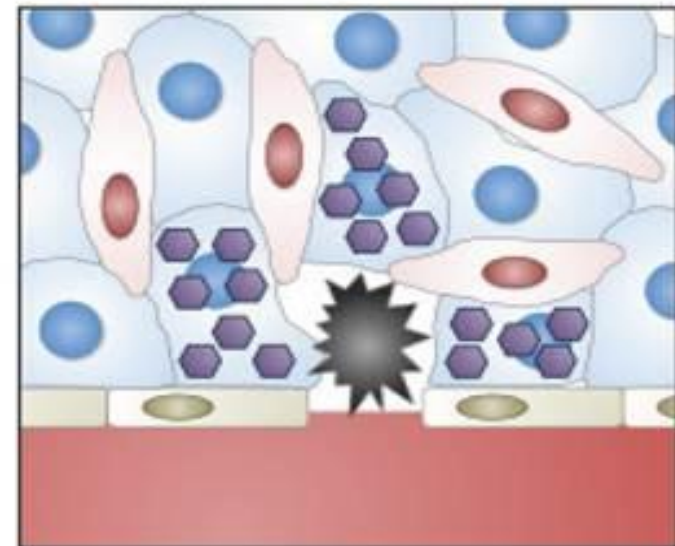
**Virus delivery through vasculature, initial infection in tumor cells, and tumor-specific replication**



**Oncolysis and spread of virus progeny**



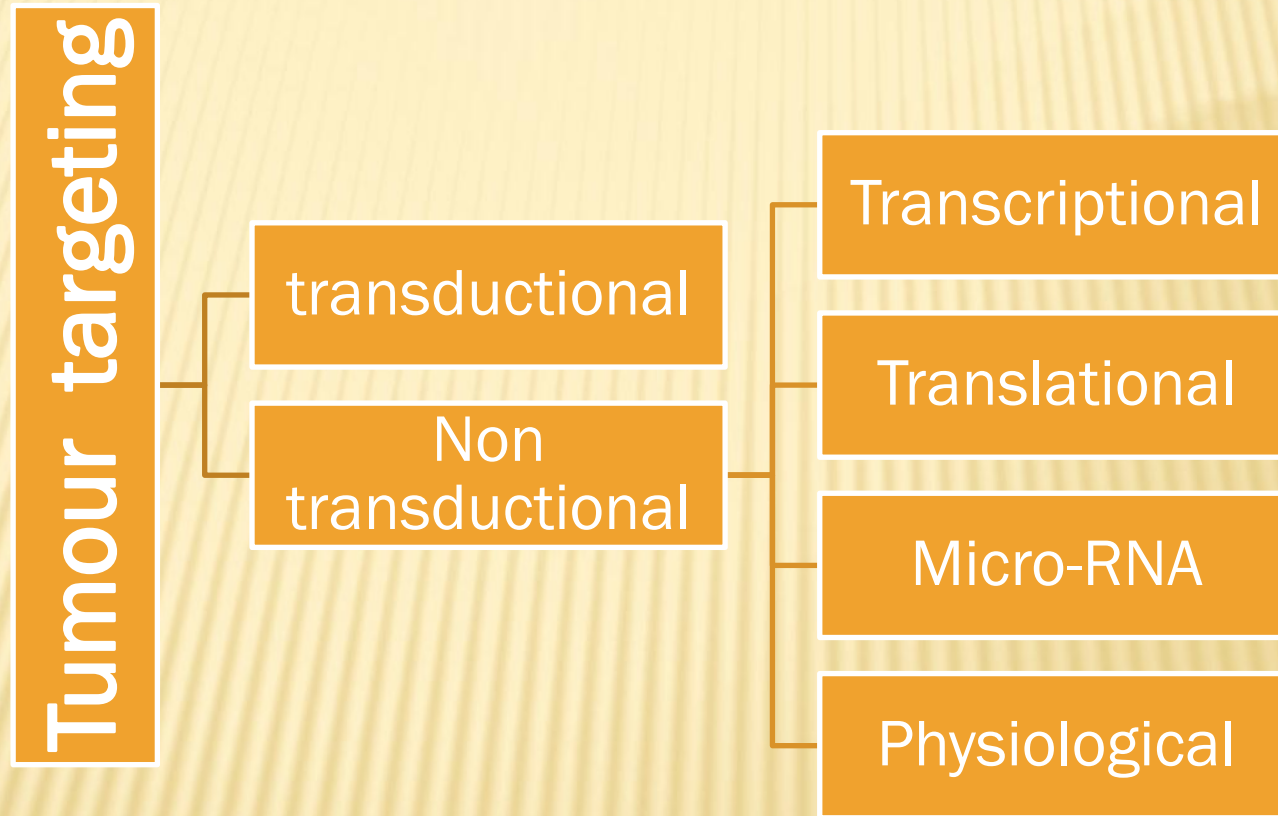
**Spread and oncolysis of tumor cells throughout the tumor while normal cells remain undamaged**



**Subsequent rounds of infection and tumor-specific replication**



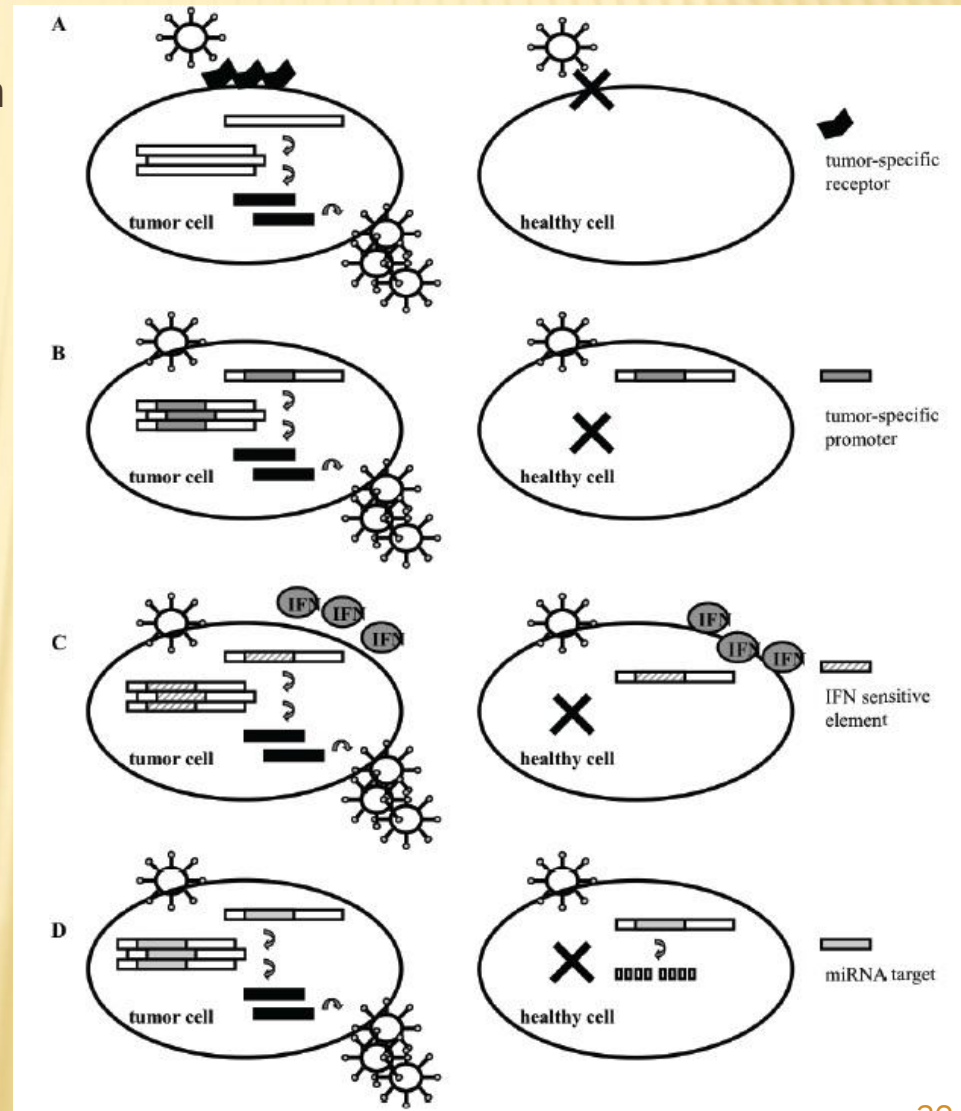
# Tumour targeting



**Double targeting** with both transductional and non-transductional targeting methods is more effective than any one form of targeting alone.

# TUMOR-SPECIFIC TARGETING STRATEGIES

- ✗ (A) **Transductional targeting**, based on the specific binding of the virus to specific or overexpressed cell membrane receptors on tumor cells
- ✗ (B) **Transcriptional targeting**, based on cell specific transcription of an essential viral gene under control of a tissue- or tumor specific cellular promoter
- ✗ (C) **Physiological targeting**, using the differences in innate immunity pathways between normal and tumor cells
- ✗ (D) **micro-RNA based targeting**, focusing on tissue-specific degradation of the viral genome in healthy cells.

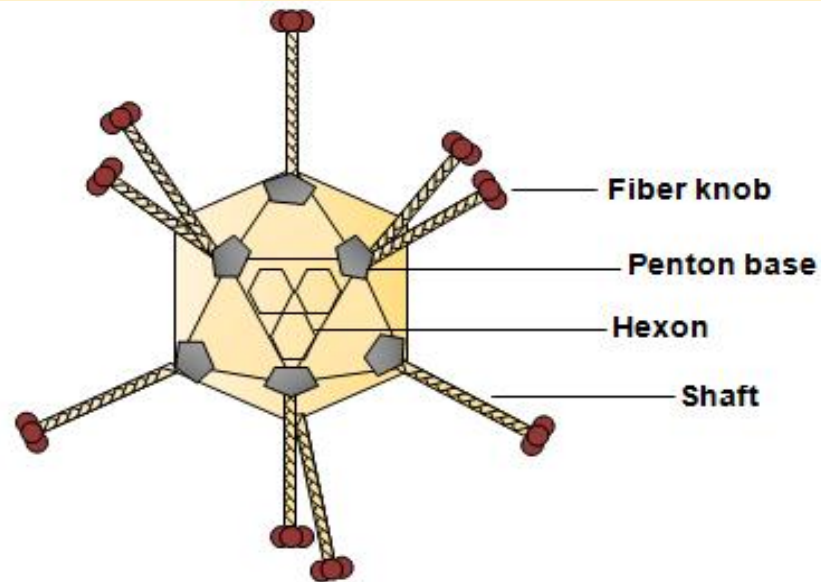




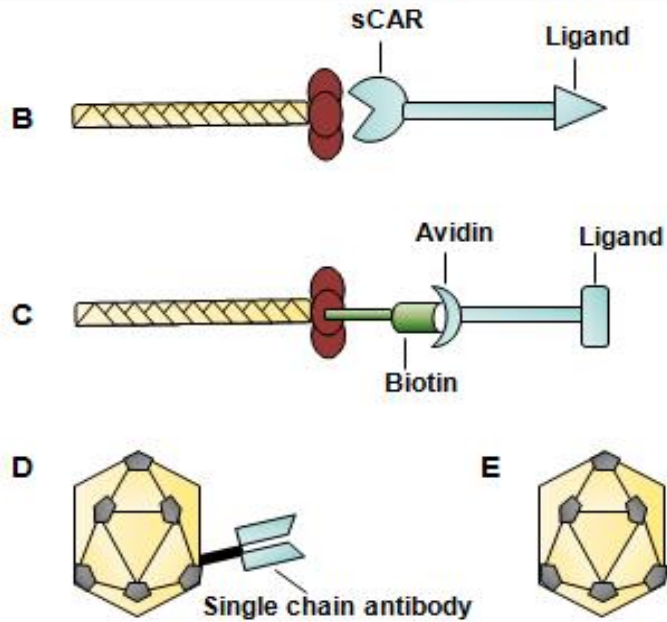
# TRANSDUCTIONAL TARGETING

- ❖ involves modifying the viral coat proteins to target tumour cells while reducing entry to non-tumour cells.
- ❖ This approach to tumour selectivity has mainly focused on adenoviruses and HSV-1, although it is entirely viable with other viruses.
- ❖ 2 main strategies:
  - changing a viral surface protein
  - using adapter proteins (which bridge the gap between the virus and the host cell)

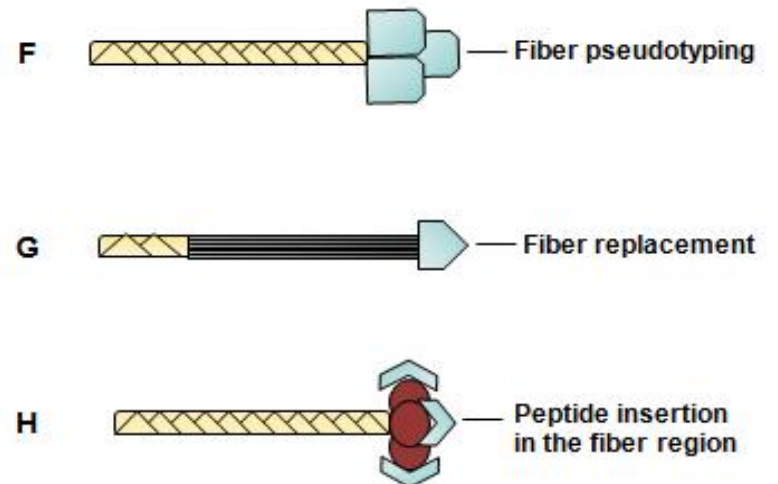
# **A Adenovirus structure**



## **Physical targeting strategies**



## **Genetic targeting strategies**





# NON-TRANSDUCTIONAL TARGETING

involves altering the genome of the virus so it can only replicate in cancer cells, most frequently as part of the attenuation of the virus

Include :

- Transcriptional Targeting
- Translational Targeting
- Physiological Targeting
- Micro-RNA Targeting

# TRANSCRIPTIONAL TARGETING

- ✗ For transcriptional targeting, which is only feasible for DNA viruses, the benefits of **tissue (or tumor) specific promoters** are exploited.
- ✗ By placing an essential viral gene downstream of such cellular regulatory element in the viral genome, viral replication can be controlled.
- ✗ Thus, progeny virus production will only be accomplished in cells expressing the selected promoter



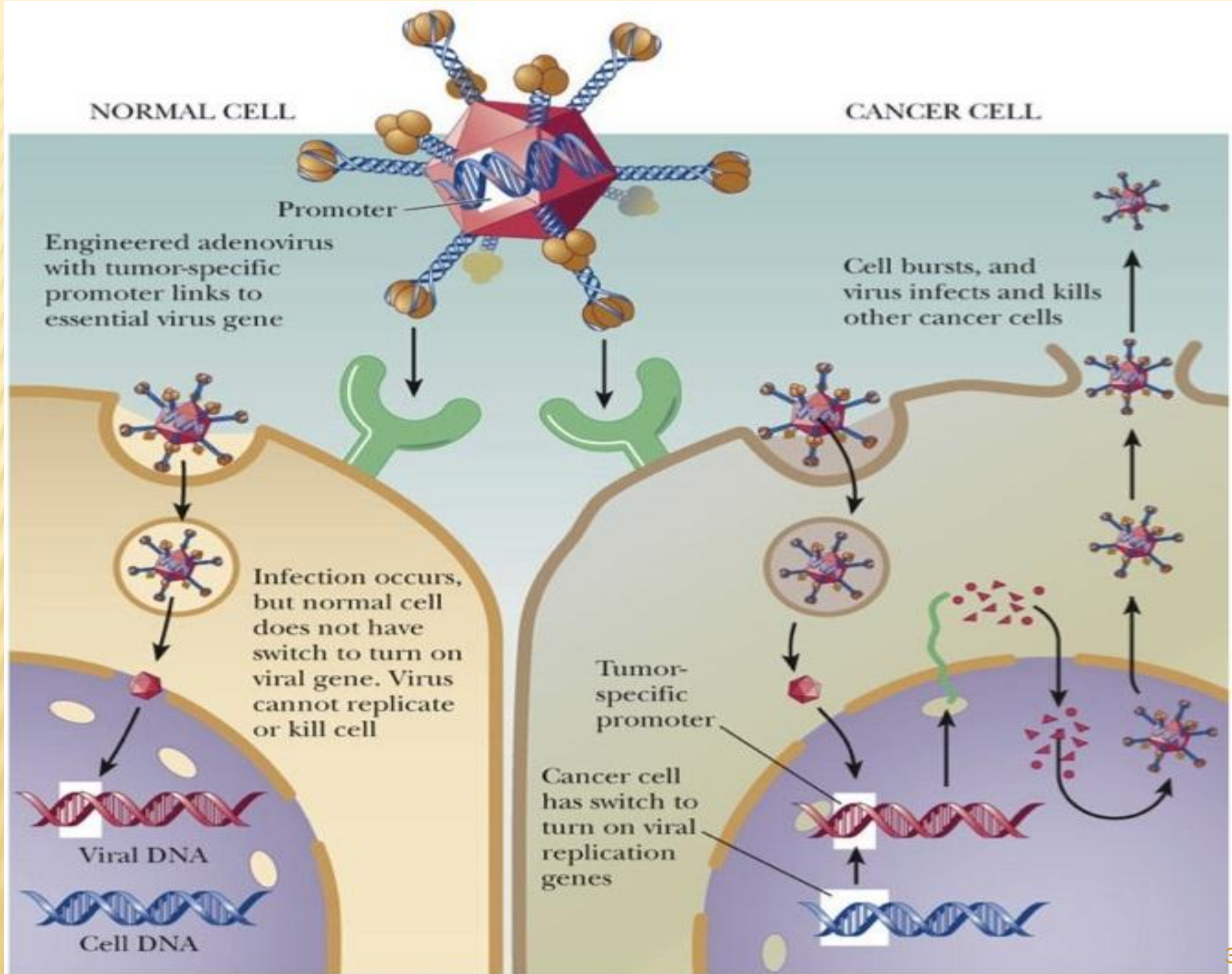
# TRANSLATIONAL TARGETING

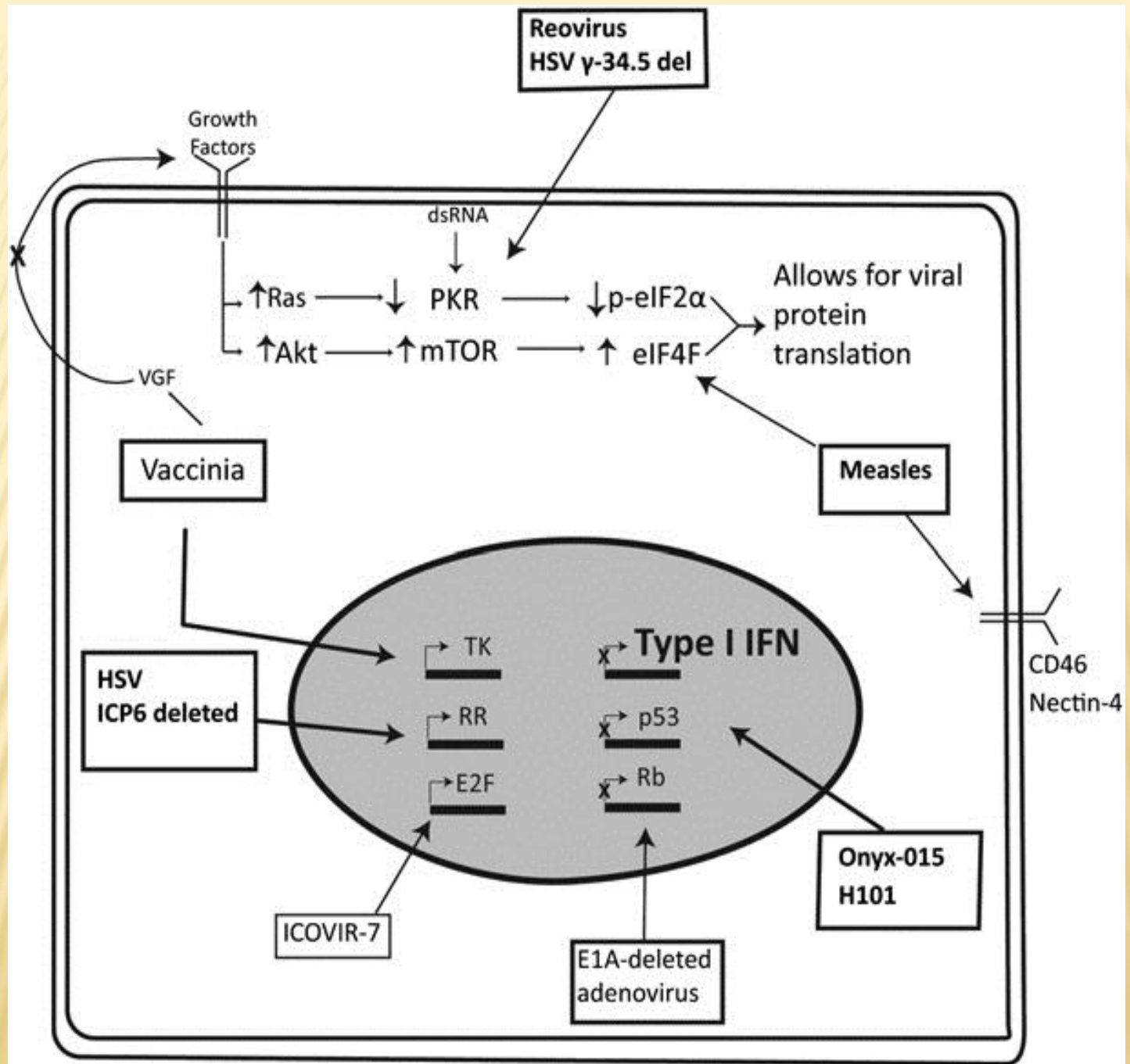
- ✗ For RNA viruses, oncolytic virus specificity can be targeted by regulation of viral protein translation.
- ✗ Inhibition of protein translation is a critical checkpoint to limit viral replication.

# TRANSCRIPTIONAL TARGETING EXAMPLE

- ✗ In one such approach, tumor-specific promoters were used to drive viral genes, as exemplified by adenovirus vectors, in which E1 gene expression is driven by the human telomerase reverse transcriptase gene (*hTERT*) that is robustly expressed in cancer but not normal cells...









# PHYSIOLOGICAL TARGETING

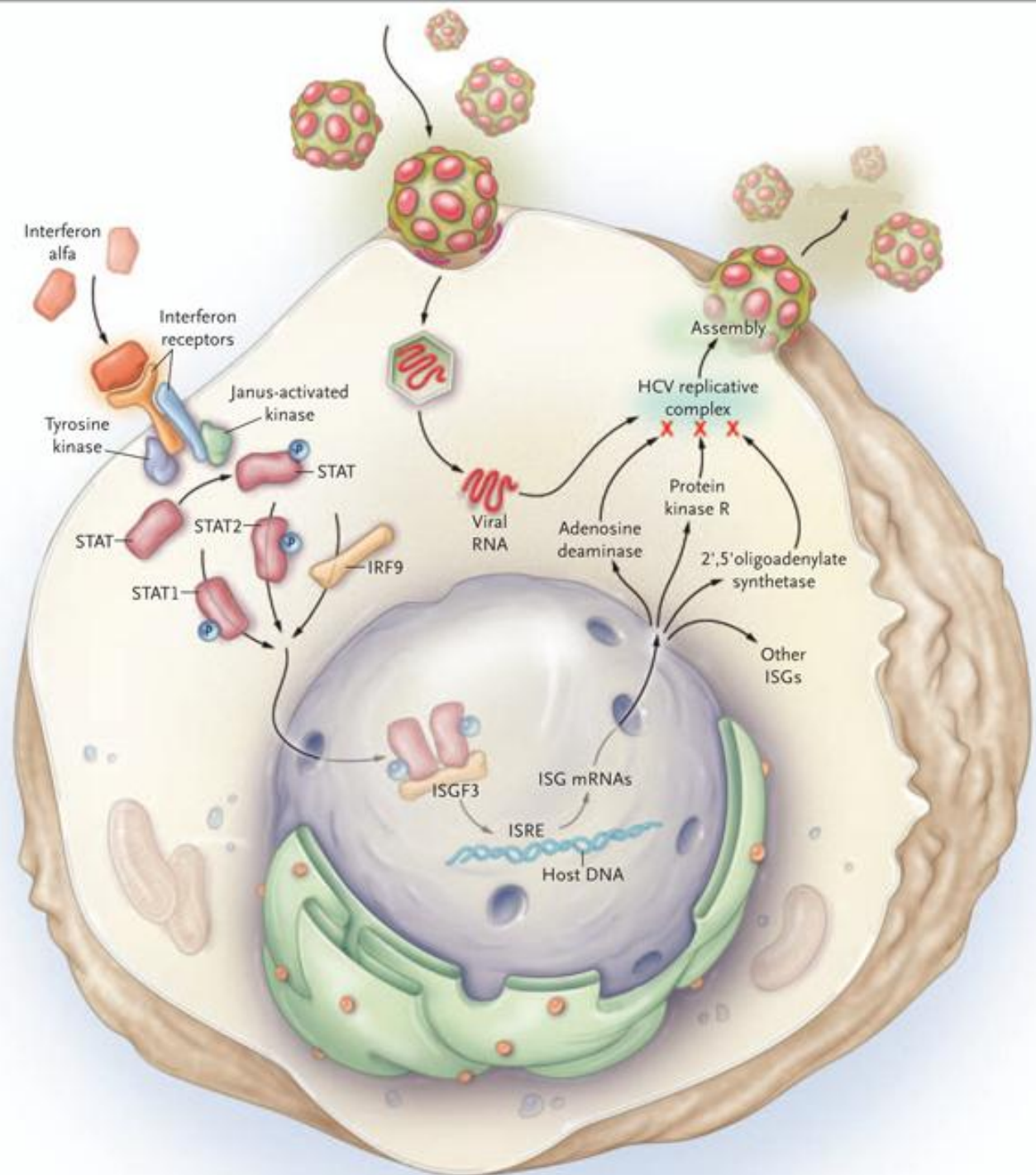
- ✖ A third strategy to increase tumor specificity exploits cancer cell defects and mainly targets the often deficient antiviral responses of tumor cells....
- ✖ The most prominent example of physiological targeting exploits the interferon (IFN) signal transduction pathway....
- ✖ Infected healthy cells often produce IFN leading to an antiviral state of neighboring cells, while tumor cells often have defects in IFN signaling pathways resulting in the lack of a proper innate immune response against the virus....

# PHYSIOLOGICAL TARGETING

- ✗ By genetic engineering of the viral genome such that immunomodulatory proteins are abolished, one can generate a virus that is vulnerable to antiviral responses in normal cells, but accomplishes replication and virus dissemination in tumor cells...



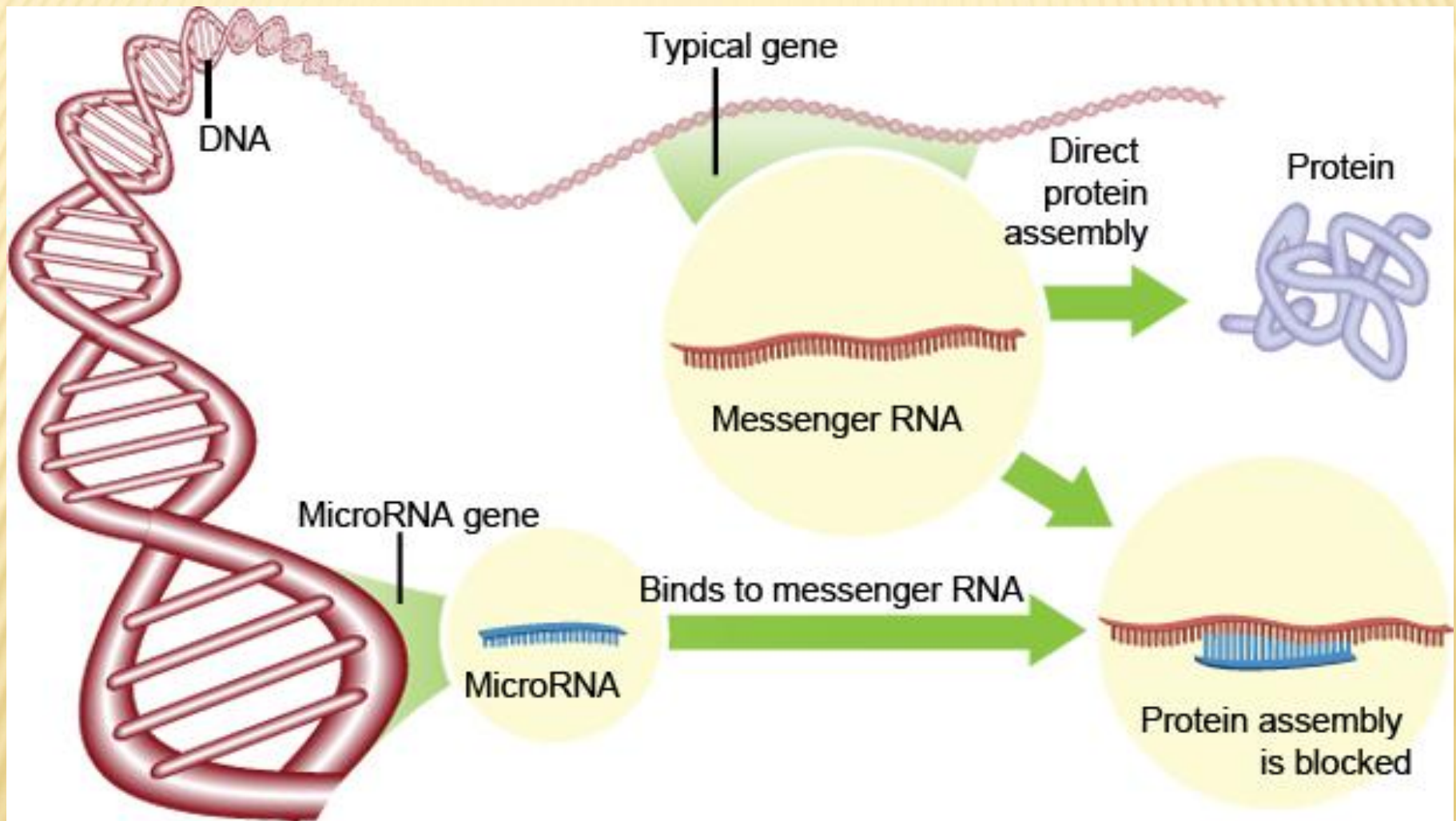
# Interferon mechanism



# MICRO-RNA TARGETING

- ✗ microRNAs manipulate the expression of genes by interacting with complementary target sequences in cellular messenger RNAs, reducing their translation or initiating their destruction.
- ✗ the most recent oncolytic virus targeting strategy focuses on the differential expression of microRNAs (miRNAs) in normal and tumor cells.





# MICRO-RNA TARGETING

- ✗ By incorporating miRNA target sequences corresponding to sequences of tissues specific cellular messenger RNAs into the viral genome, tumor-specific regulation of viral replication can be achieved...
- ✗ This strategy is used to selectively eliminate the undesirable viral tropism by preventing its replication in normal tissues...



**Table.** Clinically tested oncolytic viruses

Virus	Cancer type	Modification	Response	Reference
<b>Adenovirus</b>				
ONYX-015 H101	SCCHN Glioma Ovarian Cancer	E1B-55k deleted	PR	1-5
CGTG-401	Solid tumors	hTERT promoter CD40L expression	SD	9
Ad5/3-D24-GMCSF	Solid tumors	Ad3 fiber E1A-deleted (Rb selective) GMCSF	SD	10
Ad5-D24-GMCSF	Solid tumors	E1A-deleted GMCSF	2 CR 1 PR	11
Ad3-hTERT-E1A	Solid tumors	E1A-deleted hTERT promoter Ad3 fiber	SD	12
ICOVIR-7	Solid tumors	E1A-deleted RGD-4C modification E2F promoter HSP70 expression	1 PR	14
H103	Solid tumors		2 PR	15
<b>Herpesvirus</b>				
Oncovex-GMCSF	Breast, SCCHN, Melanoma IT Liver tumors	ICP6 deleted $\gamma$ -34.5 deleted GMCSF expression	8 CR 13 PR	21, 23, 25
NV1020	Liver tumors IA	ICP0, ICP4 deleted $\gamma$ -34.5 deleted LAT	1 PR	20
G207	Glioma IT	TK insertion ICP6 deleted $\gamma$ -34.5 deleted	8 PR	18, 19
<b>W</b>				
GL-ONC1	Solid tumors	GFP expression		Unpublished
JX-594	Liver tumors Solid tumors IV	TK deleted GMCSF expression	2 PR	26-28
Reovirus type 3 dearing	Solid tumors IV SCCHN IT	N/A	SD 3 PR	31, 32
<b>Measles virus</b>				
MV-CEA	Ovarian Ca IP	CEA expression	SD	54
MV-NIS	Ovarian Ca IP Myeloma IV Glioma IT Mesothelioma IPI	Sodium-iodide symporter expression	SD 1PR	Unpublished

**Table I** Properties of the three best-studied families of oncolytic viruses

<b>Virus type</b>	<b>Pros and cons</b>	<b>Clinical trials*</b>
Adenovirus	Pros include constructed wide host-cell range and large genomic capacity. Cons include CAR variability in human cancers and expression on normal cells, preexisting antiviral immunity, hepatic adsorption, toxicity	Completed and ongoing <sup>6,55–57</sup>
Vaccinia virus	Pros include wide host-cell range, large genomic capacity and established vaccine potential. Cons include potential difficulties with systemic delivery	Completed and ongoing <sup>4,60–63</sup>
Herpes Simplex Virus	Pros include wide host-cell range, large genomic capacity, neurotropism, ability to evade preexisting antiviral immunity and available antiviral drugs. Cons include hepatic adsorption	Completed and ongoing <sup>3,5,65–68</sup>

**Notes:** \*other viruses in clinical trials include Coxsackie virus (CVA21), measles virus (Edmonston), parvovirus, poliovirus (Sabin), retrovirus.

**Abbreviation:** CAR, Coxsackie adenovirus receptor.



# RUNAWAY FROM IMMUNITY

- ✗ viruses are rapidly cleared from the circulation as a result of sequestration by the mononuclear phagocytic system (MPS) in the liver and spleen.
- ✗ Before clearance, they are typically coated (opsonized) with antibodies, complement, coagulation factors and/or other serum proteins that facilitate their recognition by splenic macrophages and hepatic Kupffer cells..
- ✗ Also cellular immune response like interferon secreting can limite the virus...

# RUNAWAY FROM IMMUNITY

- ✖ Strategies to minimize sequestration include chemical modification of the coat proteins of the viruses by conjugation of biocompatible polymers, such as polyethylene glycol (PEG) and N-[2-hydroxypropyl] methacrylamide (HPMA).
- ✖ Both PEG and HPMA are already used clinically to prolong the circulation times of proteins and liposomes and to reduce off target toxicities.
- ✖ An alternative approach to minimize sequestration of viruses (e.g. HSV) that are readily bound by IgM and complement proteins is to deplete these serum factors by pretreating with cobra venom factor or cyclophosphamide.
- ✖ In contrast to normal cells, the successful tumor cell has often eliminated/inactivated key gene products that have the dual role of controlling critical cell growth/death programs *and* aiding in resisting virus infections. Because of these tumor-specific mutations, oncolytic Viruses can initiate productive infections in cancer cells. Occasionally, cancer cells are completely devoid of antiviral activity



## MATERIALS AND METHODS

**Cells.** Human melanoma tissue from primary and metastatic sites was isolated and up to 50% of human melanomas carry defects in this pathway (30). Patients not responding to IFN treatment might ultimately benefit from agents exploiting a defective interferon pathway in these cancer cells. A number of recombinant variants of VSV have

been constructed and tested for their ability to infect and kill melanoma cells. Even at a low viral concentration, we found a strong susceptibility to viral oncolysis in over 70% of melanomas. In contrast, melanocytes displayed strong resistance to virus infection and showed complete protection by interferon. Several recombinant VSVs were compared, and all infected and killed most melanomas with differences in

**Viruses.** Six recombinant VSV variants were used in this study: wild-type-based VSV-G/GFP, VSV-rp30, VSV-M51, VSV-CT9-M51, VSV-P126, and VSV-L223. Virus construction and characteristics of the first four have been detailed previously (34). VSV-G/GFP is based on the VSV



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# FINAL GOAL :

The ultimate challenge in the field of oncolytic virotherapy is to produce an “ideal” oncolytic therapy that is:

- ✓ **Highly selective** : able preferentially to seek and enter tumor cells while sparing non cancerous tissues and cells.
- ✓ **Replication competent**: maximally efficient in making copies of itself, amplifying in and destroying tumor cells
- ✓ **Well-tolerated**: having minimal side effects in cancer patients, and even more importantly, non pathogenic; in human patients.
- ✓ **Carries little to no risk of genomic integration** : viruses that do not integrate into the host cell DNA (replicate only in the cytoplasm) do not carry this risk, and therefore no risk of mutation.
- ✓ **Systemically administered** : in metastatic disease, the most clinically relevant approach is to target and eliminate cancer cells, tumors and metastases wherever they are located in the body.
- ✓ **Able to carry therapeutic or diagnostic payloads directly to the tumor** : specific strains of oncolytic virus can be engineered to insert and express cancer-suppressing genes and/or anti-cancer therapies.



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